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(54) Title: CCN3 COMPOSITIONS AND METHODS

(57) Abstract: CCN3 (Nov) is a matricellular protein of the CCN family, which also includes CCN1 (CYR61), CCN2 (CTGF), CCN4 (WISP-1), CCNS (WISP-2), and CCN6 (WISP-3). During development, CCN3 is expressed widely in derivatives of all three germ layers, and high levels of expression is observed in smooth muscle cells of the arterial vessel wall. Altered expression of CCN3 has been observed in a variety of tumors, including hepatocellular carcinomas, Wilm's tumors, Ewing's sarcomas, gliomas, rhabdomyosarcomas, and adrenocortical carcinomas. To understand its biological functions, we have investigated the activities of purified recombinant CCN3. We show that in endothelial cells, CCN3 supports cell adhesion, induces directed cell migration (chemotaxis), and promotes cell survival. Mechanistically, CCN3 supports human umbilical vein endothelial cell adhesion through multiple cell surface receptors, including integrins $\alpha_v\beta_3$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, and heparan sulfate proteoglycans. In contrast, CCN3-induced cell migration is dependent on integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, whereas $\alpha_6\beta_1$ does not play a role in this process. Although CCN3 does not contain a RGD sequence, it binds directly to immobilized integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ with half maximal binding occurring at 10 nM and 50 nM CCN3, respectively. Furthermore, CCN3 induces neovascularization when implanted in rat cornea, demonstrating that it is a novel angiogenic inducer. Together, these findings show that CCN3 is a ligand of integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, acts directly upon endothelial cells to stimulate pro-angiogenic activities, and induces angiogensis *in vivo*.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/09810

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C01N 33/53; A61K 49/00, 38/04; C07K 16/28
US CL : 435/7.1; 424/9.1; 530/325, 326, 327, 328, 329, 330, 388.24
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.1; 424/9.1; 530/325, 326, 327, 328, 329, 330, 388.24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, MEDLINE, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,413,735 (LAU, LF) 2 July 2002 (7.2.2002).	1-27
A	LIN et al. CCN3 (NOV) is a novel angiogenic regulator of the CCN protein family. J Biol Chem. 27 June 2003 (27.6.2003), Vol. 278, No.26, pages 24200-24208	1-27
X --- Y	SU et al. The expression of ccn3 (nov) RNA and protein in the rat central nervous system is developmentally regulated. Mol Pathol. 2001, Vol. 54, No. 3, pages 184-191.	21-22 --- 23-25
X	ELLIS et al. Nov gene encodes adhesion factor for vascular smooth muscle cells and is dynamically regulated in response to vascular injury. Arterioscler Thromb Vasc Biol. 2000 , Vol. 20, No. 8, pages 1912-1919. See page , 1913 and 1916-1917.	7 and 21-22 --- 8 and 23-25
X --- Y	GUPTA et at. Inhibition of glioma cell growth and tumorigenic potential by CCN3 (NOV). Mol Pathol. 2001, Vol. 54, No. 5, pages 293-299. See the entire document.	21-22 --- 5-6 and 23-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/09810

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: